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EPO Dir. 2.4.06 Examiner Mr. G. Wimmer

Our Ref.: **P1744PC00**
Application No.: **PCT/EP2004/000339**
Publication No.: **WO2005/068611A1**

Madrid, February 2, 2006

Re.: International PCT Application No. PCT/EP2004/000339 with title "METHOD FOR OBTAINING A SINGULAR CELL MODEL CAPABLE OF REPRODUCING IN VITRO THE METABOLIC IDIOSYNCRASY OF HUMANS", in the name of ADVANCED IN VITRO CELL TECHNOLOGIES, S.L.
Amendments and reply to Written Opinion

Dear Sirs,

This is in reply to the Written Opinion issued together with the International Search Report for PCT/EP2004/000339. In accordance with Art. 34 and Rule 66 PCT, we hereby submit a new set of claims 1-13 to replace claims 1-16 as originally filed. We respectfully request that the International Preliminary Examination be based on these amended claims.

Amendments

For the convenience of the examiner we enclose the claims with hand-written amendments and a clean copy of the new claims.

New **Claim 1** corresponds to original claim 1 with the following limitations:

- The expression vectors are now limited to "recombinant adenoviral expression vectors", this amendment is based on original claim 11 and the description page 10 lines 7-8; page 11, lines 28-29; and page 19, lines 22-23.
- The cells that are transformed have been limited to "human cells of hepatic origin", this amendment is based in part on original claim 3 and on page 8, lines 1-3.
- The expression "more than one" has been introduced to clarify that there is a plurality of expression vectors; the amendment is based on original claim 1 (plural vectors, enzymes, each coding for a different ...enzyme), and on page 8, line 6: "*one or more*", i.e. "one or *more than one*".

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Claims 2, 3, 10 and 11 have been deleted, since their subject-matter is made redundant by new claim 1.

The remaining claims have been renumbered. In new claims 3 and 5 the enzyme cytochrome c reductase has been deleted, since, as it is explained below, it is not a drug biotransformation enzyme.

New **claim 9** is a product by process claim, it is supported for the same reasons as new claim 1 and by page 6 line 6: "*A singular cell model as the one thought by this invention...*".

New **Claim 10** corresponds to original claim 13 with the following limitations:

- The expressions "more than one" and "recombinant adenoviral expression vectors" have the same basis as explained for claim 1.

- The expression "comprising ectopic DNA sequence that code for different" is based on claim 1, lines 14-15, and has been introduced for clarity purposes.

New **claim 11** corresponds to previous claim 14, only numbering and dependency have been adjusted.

New **claim 12** corresponds to original claim 15, including the expressions "more than one recombinant adenoviral" as in the other claims, and with the limitation that the expression vectors each code for a different enzyme (based on claim 1, lines 14-15).

New **claim 13** is based on original claim 16, the expression "more than one" has been introduced to clarify the plural term vectors and has basis on claim 1. Alternatives b) and c) have been deleted. The claim has been reworded for clarity reasons.

It is submitted that the amendments to the claims do not go beyond the disclosure of the International application as filed, meeting the requirements of Art. 34(2)(b) PCT.

Novelty

D1 discloses the use of human hepatocytes as a tool for studying toxicity and metabolism. D1 also discloses at pages 307-308 human hepatocyte cells transduced with adenovirus expression of transcription factors, resulting in an increase of CYP3A4 mRNA expression.

The method of present claim 1 differs from D1 in that the adenoviral expression vectors used comprise ectopic DNA sequences that code directly for different drug biotransformation enzymes, and not for their transcription factors. The cell model of present claim 9 differs for the same reasons, since it will necessarily comprise ectopic DNA coding for different drug biotransformation enzymes. The same difference applies to present independent claims 10-13. Claims 1-13 are novel over D1.

D2 describes a bacterial model (*E. coli)* genetically manipulated so that human hepatic CYPs are functionally co-expressed with NADPH-cytochrome P450 reductase. Only one CYP is introduced in each model (see page 136 and fig 1). No adenoviral expression vectors are used. Therefore present claims 1-13 differ in several features, mainly in that more than one recombinant adenoviral expression vectors coding each for different drug biotransformation enzymes are used for transforming the cells. Additionally, claims 1-9 and 11 differ in that the cell model is human and of hepatic origin.

D3 discloses the genetic manipulation of LLC-PK1, L-MDR1 and CaCo-2 cells that have been transduced with an adenoviral vector comprising human CYP3A4 cDNA. At page 807, lines 1-6 cell lines wherein both CYP3A4 and NADPH cytochrome P 450 reductase are introduced with adenovirus is described. However, the NADPH 450 reductase is not a "Phase I or Phase II drug biotransformation enzyme" but an electron donor protein for several oxygenase proteins, necessary for the metabolic activity of CYPs, but does not itself metabolize the drug.

Claims 1-13 differs from D3 in that more than one recombinant adenoviral expression vectors coding for different drug biotransformation enzymes are introduced or used. Additionally, claims 1-9 and 11 also differ in that the cell is of hepatic origin. Claims 1-13 are therefore novel over D3.

D4 describes engineered cells expressing single human CYP (see page 789). There is no mention that adenoviral recombinant vectors are used, nor of the introduction of more than one drug biotransformation enzymes. Therefore claims 1-13 are novel over D4.

D5 discloses the inhibition of CYP3A4 in human hepatocytes and on transfected CaCo-2 cell, antisense oligomers are used for the inhibition. There is no mention that adenoviral recombinant vectors are used for the manipulation of the CaCo-2 cells, nor of the introduction of more than one ectopic cDNA coding each for different drug biotransformation enzymes. Therefore claims 1-13 are novel over D4.

D6 discloses the gene transfer of β-galactosidase into human hepatocytes with adenovirus. It does not disclose the introduction of ectopic cDNA coding for more than one drug biotransformation enzymes, or in particular CYP enzymes (claim 13). Claims 1-13 are also novel over D4.

As a conclusion of the above, it is submitted that none of the documents D1-D6 discloses the subject-matter of new claims 1-13, which meets the requirement of Art. 33(2) PCT concerning novelty.

Inventive Step

We consider D1 as the closest state of the art to evaluate inventive step. D1 discusses the use of human hepatocytes as human models for evaluating toxicity and drug metabolism, and also mentions the drawbacks of these cells due to the loss of their metabolic phenotype when in culture. D1 also refers to the idiosyncratic metabolism due to high interindividual variability, see page 296, right-hand column and page 297, figure 2. According to D1 "*genetically manipulated cells expressing single CYP genes do not provide quantitative input about the participation of a given* [drug biotransformation] *isoenzyme in the overal metabolism of a drug*", due to the fact that "*in cDNA expressing systems a single CYP interacts with an electron-carrier/supplier protein, while in liver hepatocytes many CYPs can interact with them. This can lead to incorrect predictions of the relative contributions of individual CYPs to the metabolism of a drug.*" (page 299, left-hand column, lines 44-65).

The problem to be solved over D1 can therefore be seen as to provide a singular cell model capable of reproducing *in vitro* the metabolic idiosyncrasy of humans, i.e. able to maintain different levels of expression of different Phase I or Phase II drug biotransformation enzymes, these levels being modullable in order to reproduce the interindividual variability (see present application, page 4, lines 9-23).

The present invention provides the solution to this problem: transforming the cells with a set of more than one recombinant adenoviral expression vectors comprising each an ectopic DNA sequence that code for different drug biotransformation enzymes. This allows to modulate at will the level of expression of each drug biotransformation enzyme, and to reproduce the diferent patterns of idiosyncratic metabolism. The inventors have found that several drug biotransformation enzymes can be introduced simultaneously in a cell model, and that the level of expression of a particular drug biotransformation enzyme depends linearly of the amount of recombinant adenovirus used for the infection. This results in a easy modullation of the expression level of each enzyme. Further, there are no significant alterations in any other function of the cells. Thus, singular cell models can be generated with specific phenotypes by only linearly varying the concentrations of the expression vectors used to transform the cells (page 11, lines 13-18).

Neither D1, nor any of the cited documents, suggests that the problem posed could be solved this way.

D1, at pages 307-309 suggests the infection of a hepatoma cell line with adenoviral recombinant expression vectors introducing transcription factors in order to re-express relevant CYPs. However, the person skilled in the art will recognise that this is not a suitable approach, because transcription factors are involved in a cascade of events, affecting several enzymes at the same time. This approach does not allow a controlled modulation (increase or decrease) of the levels of expression for each drug biotransformation enzyme.

D3 is concerned with the introduction of CYP3A4 and transport proteins activity to certain cells. The strategy used is infection of the cells with an adenoviral recombinant expression vector carrying CYP3A4 cDNA.

However, D3 teaches away from the invention, since the level of expression of CYP3A4 varies significantly depending on the cell models which is infected (see pages 805-806 and figure 1), those of human origin he uses (CaCo-2) are transduced with a much lower efficiency. Although D3 mentions a certain dose dependency, this dependency is variable, not linear, and depends on the cell type being infected.

Further, there is no indication in D3 that the introduction of more than one adenoviral recombinant vectors comprising cDNA encoding for different drug biotransformation enzymes would not affect the levels of expression among them. On the contrary, D3 shows that when adenovirus carrying CYP3A4 and adenovirus carrying NADPH cytochrome P450 reductase are used simultaneously to infect the cells, a synergistic effect is obtained on the level of expression of CYP3A4 (see table 3). Thus, starting from D1 and faced with the problem to be solved, there is no suggestion in D3 that would direct the person skilled in the art to infect the cells with more than one adenoviral expression vectors carrying different drug biotransformation enzymes, because he would not expect to be able to modullate them. On the contrary, he knows from D3 that when introducing more than one enzyme he can expect interactions between the levels of expression of the enzymes. D3 does not teach or suggests how to manipulate at will easily and simultaneously the levels of the various drug biotransformation enzymes of a human cell.

Documents D2 or D4 do not provide the solution either, because they disclose genetically manipulated cells where only one CYP gene is introduced at a time. This imply that instead of one single model able to reproduce the metabolic indiosyncrasy, they provide a set of x models, one for each enzyme. Moreover, they do not control the levels of expression for each enzyme. In

fact, D4 already states the limitation of these models: "*By comparing the data on metabolic rates obtained with CYP-expressing cells and human hepatocytes, an estimation of the degree of participation of each CYP in the metabolism of diclofenac in the human liver can be made*". (sentence bridging pages 794-795).

In conclusion, we submit that the invention, as defined in present claims 1-13, is not obvious for a person skilled in the art having regard to the prior art, and therefore meets the requirements of Art. 33(3) PCT.

Based on the arguments and amended claims, we respectfully request the examiner to reconsider the Written Opinion. We believe there is now basis for a positive IPER. If necessary, the undersigned is available for a telephonic consultation.

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Encl. -Amended claims (clean copy and with hand-written amendmends)